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The prophylactic efficacy of Cardamom (*Elettaria cardamomum*) extract against aspirin-induced gastric ulcer in a rat model

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ABSTRACT

The gastroprotective and therapeutic effects of cardamom was examined using aspirin-induced gastric ulcers. Male albino rats were separated into healthy control and peptic ulcer-induced groups. The latter group was treated with cardamom extract before or after ulcer induction to investigate its protective and therapeutic effects. Another group was treated with Omeprazole. Rats were sacrificed; the gastric tissue was examined for ulcer index and score, pH and total acidity measurements. Moreover, we studied anti-inflammatory and antioxidant biomarkers. Results demonstrated that cardamom exerts an ameliorative effect, shown by a decrease in the gastric ulcer index and score. The extract restores the free radical-scavenging enzymes and reduces inflammatory markers. Pepsin and putrescine levels decreased significantly, while hydroxyproline levels increased in groups treated with cardamom extract, either as a protective or therapeutic agent. Cardamom is therapeutically valuable for enhancing and hastening gastric ulcer remediation as it is capable of inhibiting aspirin-induced damage.

Key Words: Peptic ulcer, Cardamom extract, ulcer index, anti-inflammatory, antioxidant.

1. INTRODUCTION

Gastric ulcer, a disease that afflicts people around the world, occurs because of an imbalance between the 'aggressive' and 'protective' agents acting on the inner epithelial cell surface (Awaad et al., 2013). The condition occurs when the balance between the damaging effects of gastric acid, pepsin, ischaemia, *Helicobacter pylori*, bile acids, nonsteroidal anti-inflammatory drugs (NSAIDs), smoking and alcohol and the protective mechanisms, including mucus layer, bicarbonate, growth factors and mucosal blood flow, which preserves the gastric mucosa, is disrupted. The hazard of epithelial erosion, following ulcer development, occurs when the reparative and protective mechanisms decline and/or if the acid–pepsin mixture is strong and continues for a long duration (Asmari et al., 2014).

NSAID usage is not recommended for elderly individuals, cardiac patients, people with a history of ulcers and those using anticoagulants. Patients who consumed ulcer-inducing NSAIDs should take a proton pump inhibitor, such as Omeprazole, which binds to the H^+/K^+-ATPase enzyme system (proton pump) of the parietal cells, overwhelming the release of hydrogen ions into the gastric lumen (Das et al., 2012).

Small ulcers have no symptoms, but some larger ones can cause bleeding. Other symptoms include a sensation of fullness, inability to drink plentiful fluids, hunger and mild nausea. Moreover, bloody stools, fatigue, chest pain, weight loss and vomiting are also likely to occur (Awaad et al., 2013).

Various studies have been conducted to identify natural and novel anti-ulcer treatments to substitute for presently used medications, which offer uncertain efficiency and protection (Elinav et al., 2013). In the scientific literature, several medicinal herbs and their metabolites have been recommended because of their antiulcerogenic properties; these were either due to prophylactic or therapeutic mechanisms or, at times, both (Kwiecien et al., 2002). Herbs and dietary supplements were encouraged as they are cost-effective, harmless and beneficial methods with preventive effects. In addition, these dietary interventions offer protection against several other diseases (Pillai et al., 2010).

Cardamom (*Elettaria cardamomum*) is a popular green species containing phenolic acids, volatile oils, sterols and lipids(Joshi et al., 2013).Green cardamom has been used traditionally to treat diarrhoea, dyspepsia, hypertension, epilepsy, gastrointestinal disorders, vomiting, ulcers and cardiovascular diseases (Gilani et al., 2008). Moreover, the herb stimulates the metabolism and exhibits antioxidant and anti-inflammatory effects.

Cardamom oil contains 50% 1,8-cineole, with trace amounts of citronellol, terpineol, myrceneborneol, phellandrene and pinene (Amma et al., 2015). The herb exhibits anti-inflammatory, anticancer, gastroprotective, antihypertensive and immunomodulatory properties (Goyal et al., 2015).

Animal studies have established that cardamom has antioxidant effects in addition to having antihypertensive, gastroprotective, spasmolytic, anti-bacterial, anti-platelet and anticancer properties (Pohle et al., 2001).

In this study, we explore the potential effects of cardamom extract through using anti-inflammatory, antioxidant and gastroprotective biomarkers in peptic ulcer experimental model.

2. MATERIALS & METHODS

Drugs and Chemicals

Aspirin and Omeprazole were purchased from Sigma Aldrich.

Experimental Animals

Forty adult male albino rats, of Wistar strain (weighing: 120–130 g), were obtained from the Animal House Colony of the King Fahd Medical Research Centre (KFMRC) and acclimatised to a temperature of 25 ± 1ºC and humidity of 55%. The rats were controlled with 12 h light/dark cycles at KFMRC's Animal Facility Breeding Colony and maintained with *ad libitum* access to a standard pellet diet and tap water. The animals were cared for according to the guidelines for animal experiments, which were approved by the Ethical Committee of KFMRC, Jeddah, KSA. Approval number (163-18).

Experimental Design

After the acclimatisation period (one week), the animals were randomly assigned to five experimental groups (eight rats/group). The first group was set as negative control and received 1 mL of saline daily. In the second, peptic ulcer-induced group (positive control), gastric ulcer was induced in fasted rats by oral administration of aspirin (single dose 500 mg/kg body weight) (Verma & Kumar, 2016). In the third group (prophylactic group): protection from peptic ulcer was provided with 2% cardamom powder, mixed with the diet, for two weeks before induction and one week after induction. In the fourth group, the ulcer was first induced and then the

rats were fed a diet containing 2% cardamom (Darwish and Abd El Azim, 2013). In the fifth group (therapeutic group), the peptic ulcer was treated with omeprazole (reference drug) at a dose of 20 mg/kg body weight. After completing treatment, the animals were fasted overnight, and blood samples were withdrawn from the retro-orbital venous plexus under diethyl ether anaesthesia, left to clot, separated by centrifugation at 4ºC at 1800× *g* for 10 min, and stored immediately at −20ºC in clean plastic Eppendorf tubes until required for biochemical analysis.

Preparation of the plant extract

Dry cardamom seeds were purchased from the local market and made into a fine powder with a pulveriser. This powder was taken in a mortar and 2% gum acacia was added, little by little, and triturated continuously to obtain a suspension (Goyal et al., 2015).

Biochemical analysis

Gastric juice parameters: The stomach was opened along the greater curvature, and the gastric contents were drained into a centrifuge tube. The gastric juice was centrifuged at 1000 rpm for 10 min (4°C), and the clear supernatant was used for the analysis. Gastric pH was measured with a pH meter. To calculate the ulcer index (UI), the rat's stomach was washed with saline, after draining the juice and examined by a magnifying lens. The score was calculated as the mean number of ulcers in each group (total number of ulcers divided by the number of rats) The UI was then derived by multiplying the ulcer score by 100. Gastric acidity was measured using a Denver instrument.

Hydroxyproline was determined using enzyme-linked immunosorbent assay (ELISA) kit, Cloud-clone Corp, TX, USA. Total polyamine (Putrescine) was estimated fluorometrically with Biovision Kits. Pepsin was measured using ELISA (Kamiya Biomedical Co., CA, USA).

The inflammatory biomarkers of interleukin-6 (IL-6), interleukin-10 (IL-10) and C-reactive protein (CRP) were measured using ELISA, Kamiya Biomedical Co., CA, USA. Antioxidant status, including malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC) and xanthin oxidase (XO) were evaluated spectrophotmetrically using Biovision Kit, CA. USA.

3. RESULTS

Cardamom extract protective and therapeutic effects on gastric ulcer biomarkers are provided in Table 1. Our results asserted a significant elevation in the peptic ulcer score and index, as well as total acidity, in the peptic ulcer-induced group compared to the control group. The gastric juice pH showed a significant reduction in the peptic ulcer-induced group ($P \leq 0.01$). Treatment with cardamom extract, either as protective or therapeutic agent, resulted in a significant regression in the ulcer score, UI and total acidity compared to the peptic ulcer-induced group ($P \leq 0.01$). The gastric injury biomarkers in the cardamom extract group were quite similar to those of the Omeprazole treated group.

Table 2 provides the pepsin, hydroxyproline and putrescine levels in all the investigated groups. It is clear from these data that the levels of pepsin & putrescine were remarkably increased in the peptic ulcer-induced group compared to the Omeprazole and cardamom extract treated groups ($P \leq 0.01$). On the other hand, hydroxyproline decreased significantly in the peptic ulcer-induced group compared to the other groups ($P \leq 0.01$).

Oxidative stress status is represented in Table 3. It could be inferred that the peptic ulcer-induced group displayed a significant decrease in SOD, TAC and XO compared to the healthy control group ($P \leq 0.01$). Besides, we found a significant increase in the MDA level in the peptic ulcer-induced group that was not apparent in the other groups. Hence, the cardamom extract appears effective in alleviating antioxidant status in the treated group ($P \leq 0.01$).

Regarding inflammatory biomarkers, Figures (1,2,3) results confirmed a significant elevation of the pro-inflammatory biomarkers of IL-6 and CRP, accompanied by a significant regression of the anti-inflammatory IL-10 in the peptic ulcer-induced group compared to the healthy control group ($P \leq 0.01$). Treatment with cardamom extract, either as a protective of therapeutic agent, resulted in a significant amelioration of inflammatory biomarkers compared to the peptic ulcer-induced group ($P \leq 0.01$). Results of the cardamom extract closely resemble those of the reference drug (Omeprazole) used in this study.

Table 1 Protective and therapeutic effect of cardamom extract on gastric biomarkers in all investigated groups. (Mean ± SD)

Groups	Ulcer score	Ulcer index%	Gastric juice pH	Total acidity (Meq/l)
Control	0	0.0000	3.4±0.06	41.6±2.9
Peptic ulcer (PU)	28.5± 1.6*	2850±164*	2.3±0.09*	76.1±1.8*
PU + cardamom (protective)	16.6±1.36**	1666±136**	2.8±0.05**	57.2±1.7**
PU + cardamom (therapeutic)	21.6±0.89**	2100±89**	2.6±0.09**	71.4±1.45**
PU + Omeprazole	11.4±0.89**	1150±54**	3.15±0.07**	50.8±2.6**

*means ±SD are significant (P<0.01) compared with normal control group (1), **means ± SD are significant. (P < 0.01) as compared with the ulcer group (2).

Table 2 Protective and therapeutic effect of cardamom extract on pepsin, hydroxyproline and putrescine in all investigated groups. (Mean ± SD)

Groups	Pepsin (µg/ml)	Hydroxy Proline (ng/ml)	Putrescine (nmol/ml)
Control	122.7±2.2	159.8±4.9	8.1±0.2
Peptic ulcer (PU)	346.3±11.4*	107.1±4.1*	11.4±1.0*
PU + cardamom (PU protective)	224.6±9.3**	135.8±3.8**	9.4±0.25**
PU + cardamom (PU therapeutic)	314.6±9.77**	124.8±1.6**	9.8±0.82**
PU + Omeprazole	205.7±4**	144.5±2.3**	8.5±0.39

**means ± SD are significant (P < 0.01) as compared with normal control group (1), **means ± SD are significant, (P < 0.01) as compared with the ulcer group (2).*

Table 3 Protective and therapeutic effect of cardamom extract on oxidative stress biomarkers in all investigated groups (Mean ± SD)

Groups	SOD (U/ml)	MDA(nmol/ml)	TAC(mmol/ml)	X-Oxidase (mU/ml)
Control	13.2±0.87	3.69±0.27	26.95±2.9	68.80±1.5
Peptic ulcer (PU)	5.80±0.7*	7.12±0.84*	12.45±0.63*	44.18±4.3*
PU + cardamom (PU protective)	9.98±0.72**	5.55±0.3**	17.35±0.87**	61.11±1.2**
PU + cardamom (PU therapeutic)	7.66±0.35**	6.21±0.84*	14.98±0.8*	52.78±1.8**
PU + Omeprazole	11.58±0.99**	4.360±0.30**	21.24±1.4**	64.70±0.66**

**means ± SD are significant (P < 0.01) compared with normal control group (1), **means ± SD are significant (P < 0.01) as compared with the ulcer group (2).*

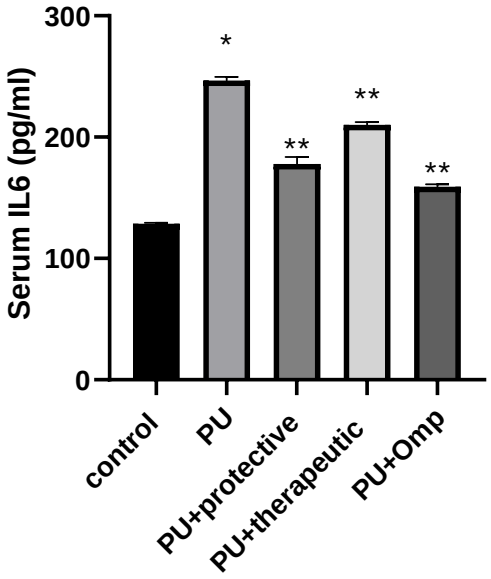

Figure (1):Effect of different treatments on serum IL6 (pg/ml).
***means ± SD are significant (P < 0.01) as compared with the normal control group (1)**
, ****means ± SD are significant (P < 0.01) as compared with the ulcer group (2).

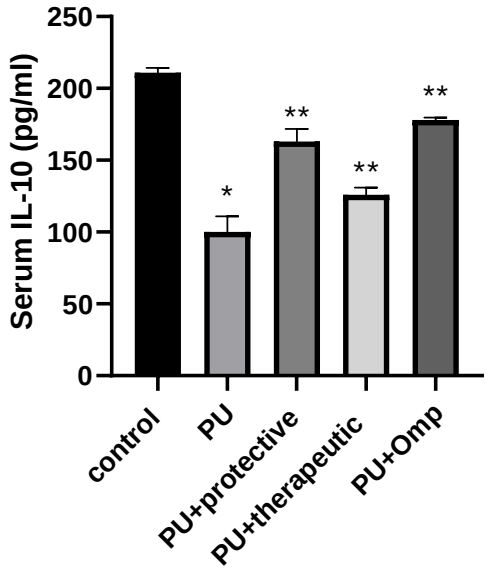

Figure (2):Effect of different treatments on serum IL-10 (pg/ml).
***means ± SD are significant (P < 0.01) as compared with the normal control group (1)**
, ****means ± SD are significant (P < 0.01) as compared with the ulcer group (2).

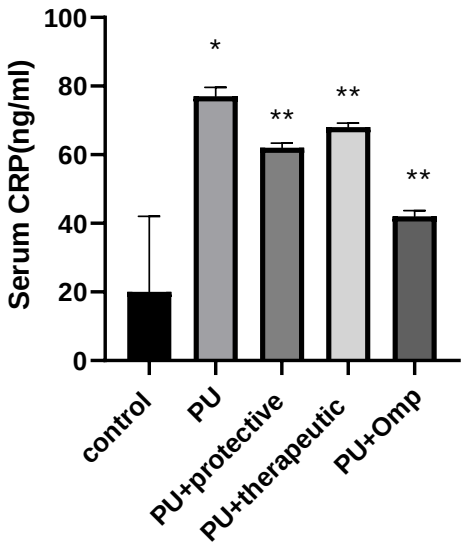

Figure (3):Effect of different treatments on serumCRP(ng/ml)
***means ± SD are significant (P < 0.01) as compared with the normal control group (1)**
, ****means ± SD are significant (P < 0.01) as compared with the ulcer group (2).

4. DISCUSSION

Gastric ulcer is a common illness affecting millions of individuals; hence, research on remedial processes is pertinent. The condition is well-known as a mucosal breakdown, with considerable histological signs in the submucosa. Different reasons, such as *Helicobacter pylori* infection and hydrochloric acid secretion, affect the gastric mucosal walls and lead to ulcers (Awad et al., 2013).

In this study, aspirin was used to induce gastric ulcers. This induction could be explained by different mechanisms, including enhanced stomach permeability, prostaglandin synthesis inhibition, mitochondrial dysfunction, or a combination of any two mechanisms (Alabi et al., 2017). Aspirin harms the gastric mucosa as shown previously in animals and humans studies due to mucosal damage that manifested by hemorrhage and erosion (Musalmah et al., 2002). Gastrointestinal problems were most important side effects of aspirin usage (De Abajo and Garcia 2001).

Previous investigations have revealed that aspirin increases the ulcer score, UI and total acidity, and it has been implicated in lowering the gastric juice pH, and raising the volume of the gastric juice, or declining the volume of the juice and its acid output. These changes were also reported by Wang et al (2007). However, a study by Al-Mofleh, (2010) showed that peptic ulcerations and gastrointestinal bleeding were the main causes of aspirin limitation in clinical applications. Due to acidic PH of stomach, aspirin finds an easy contact to the inner epithelial cells wherein it surrounded causing mucosal injury, changes mucosal wall function and reduces hydrophobicity of epithelial surface (Halter et al., 2001).

Using cardamom in this study as protective agent significantly reduced ulcer score compared to ulcer group, associated with its healing effect on mucosal integrity, acidity and gastric juice PH. Parallel results were detected by Vasudevan et al., (2000). Cardamom protects gastric mucosa by inhibition of gastric acidity that reduces ulcer formation (Arawwawala et al., 2010). On the other hand, Omeperazole group showed improvement in markers of gastric ulcer, Omeprazole is the most commonly prescribed proton pump inhibitor (PPI) for gastroesophageal hyperacidity, gastric ulcers, duodenal ulcers, and reflux esophagitis (Targownik et al., 2007).

Aspirin induced ulcer was accompanied with elevation in pepsin and putrescine, gastric acid secretion is encouraged by releasing histamine from oxyntic glands (Biondo et al., 2011). On the other hand, there was a noticed reduction in hydroxy proline in ulcerated rats, this may due to reduction in collagen in the stomach, as hydroxyproline is an amino acid that found in collagen and elevate collagenase enzyme activity and reduced levels of collagen in stomach distressed with aspirin-induced ulcers was reported by Takeuchi et al., (2013). In a study by Pradeepkumar et al., (2011) an elevation in putrescine was also obviously detected in the aspirin-induced ulcer rats.

Oxidative stress is an important cause of numerous diseases, particularly gastric ulceration. Studies clarified aspirin's effects on free radical and oxidative stress parameters, as the drug caused mucosal erosion by elevating lipid peroxidation (Ibrahim et al., 2016). These results are concordant with previous data, in which the ulcer reduced SOD, TAC and XO levels and increased the MDA

level. While Omeprazole treatment returned the oxidative biomarkers close to their normal levels, protection using cardamom also partially modified the gastric oxidative biomarker levels, with a significant change compared to the ulcer group. The reactive oxygen species (ROS), produced in the lumen targets, the gastric mucosal epithelium, causing obvious cell damage by interrelating with the DNA, proteins and lipids, thereby promoting lipid peroxidation(Viana et al., 2013).

The ROS mechanism causes epithelial cell erosion and enhances inflammation, accentuating gastric ulcer bleeding due to acidic gastric juice overproduction (Hamauzu et al 2008). Aspirin increases ROS levels and reduces mucus viscosity, thereby causing further tissue damage (Rao and Venkataramana, 2013). MDA is the main product of lipid peroxidation, so its measurement can indirectly indicate the level. Olaleye and Akinmoladun (2013) discerned an association between gastric ulcer and elevated MDA level. These data are supported by the results of Sidahmed et al. (2015) Decreased SOD activity indicates damage to the cell's protective mechanisms (Kwiecien et al., 2014). The results clearly showed that, while aspirin altered the oxidative status and decreased the protection of the gastric mucosa, cardamom extract offered protection against the induced ulcer. The herb has a strong antioxidant potential (Shahidi and Ambigaipalan, 2015). Numerous studies have indicated cardamom extract has anti-inflammatory, antiproliferative, pro-apoptotic and antioxidant activities (Das et al., 2012). Goyal et al. (2015) stated that the active constituents in cardamom protect against myocardial infarction by reinstating cellular antioxidants. Kaur et al. (2008) established that drinking cardamom-containing green tea reduces the enzymatic conversion of SOD and MDA products. Cardamom seed powder is often used to treat gastrointestinal illnesses and is used as a carminative, resolvent, retentive, digestive, stomachic and antiemetic agent. The use of herbs in treating acid peptic disorders and gastritis has been recommended (Malagelada et al., 2007). Cardamom's power may be due to the antioxidant compounds in its essential oil, such as gallic and tannic acids(Sarayana and Geetha, 2011).

Omeprazole treatment corrects the MDA, TAC and SOD gastric levels because of its antioxidant characteristics, and it reduces lipid peroxidation by hydroxyl radical scavenging (Biswas et al., 2003). Moreover, studies have recognised the drug's capability to raise the gastric nitric oxide level and protect the gastric tissue in case of alcohol-induced ulcer (Rouhollahi et al., 2014).

Inflammation refers to an increase in the vascular tissue surface area because irritation is caused by pathogen or injured cells. Gastric immune cell changes may be concerned with the GU pathophysiology as cytokines and neuroactive chemicals produced by the immune cells may change the gastrointestinal sensitivity (Lee et al., 2013).

Results of inflammatory biomarkers indicated increased IL-6 and CRP levels, with a concomitant reduction of IL-10 levels in ulcerated rats. A study by Raghavendran et al., (2011) testified that aspirin-induced ulceration in rats enhanced cytokine production as indicated by increased IL-6 and IL-10 levels. Gastric mucosal inflammation and neutrophil infiltration are related to aspirin-induced gastric ulceration (Alabi et al., 2017). There were two reasons that account for the drug's side effects. First, aspirin inhibits cyclooxygenase-1 (COX-1), an enzyme mainly expressed in gastric epithelial cells (Rouhollahi et al., 2014). Second, the compound possesses a free carboxylic group that can also cause an irritant effect on the gastric mucosa (Amagase et al., 2013). Large numbers of neutrophils and macrophages enter the wounded mucosa during acute inflammation, causing ROS production and leading to mucosal damage and bleeding (Syam et al., 2009).

Cardamom administration as a protective agent could modulate inflammatory biomarker levels. The herb is also capable of inhibiting colon and skin cancer because of enhanced antioxidant activity (Majdalawieh and Carr, 2010). Cardamom seed extract also has anti-inflammatory properties (Majdalawieh and Carr, 2010). Pillai et al., (2010) demonstrated that α-phellandrene (the main component of cardamom) inhibited production of the pro-inflammatory cytokines TNF-α and IL-6 in mice. Omeprazole also exhibited an anti-inflammatory effect by causing a substantial reduction of inflammatory biomarkers. Abood et al., (2014) reported that Omeprazole administration caused a significant reduction in inflammatory biomarkers in alcohol-induced ulcer.

5. CONCLUSION

Our results strongly indicate that aspirin-induced gastric ulcer is associated with gastric mucosal damage and a significant disturbance to the antioxidant status and inflammatory biomarkers. Such deleterious effects were effectively eliminated by the oral supplementation of cardamom.

Conflict of interest

Authors declare that no conflict of interest

Financial resources of the study

None

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